UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 15, 2008

MEMORANDUM

Subject: Efficacy Review for DisCide ULTRA Disinfecting Spray, EPA Reg. No. 10492-5; DP Barcode: D357772.

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1/16/09

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To: Marshall Swindell / Martha Terry
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Applicant: Palmero Health Care
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Formulation from the Label:

Active Ingredient	% by wt.
n-alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride	0.12 %
n-alkyl (68% C_{12}, 32% C_{14}) dimethyl ethyl benzyl ammonium chloride	0.12 %
Isopropyl alcohol	63.25 %
Inert Ingredients	36.51 %
Total	100.00 %

I. BACKGROUND

The product, DisCide Ultra Disinfecting Spray (EPA Reg. No. 10492-4), is an EPA-approved disinfectant (bactericide, tuberculocide, virucide, fungicide) for use on hard, non-porous surfaces in institutional, commercial, food service, animal care, and hospital or medical environments. The applicant requested to amend the product registration to add claims for effectiveness as a disinfectant against *Candida albicans* and *Proteus mirabilis*. The label claims a reduced contact time against Avian Influenza (H3N2) virus, Influenza A virus, and Human Coronavirus. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121

This data package contained a letter from the applicant representative to EPA (dated September 29, 2008), EPA Form 8570-35 (Data Matrix), five studies (MRID 475543-01 through 475543-05), Statements of No Data Confidentiality Claims for all five studies, and the proposed label (dated September 29, 2008).

II. USE DIRECTIONS

The product is designed for use in disinfecting hard, non-porous, non-food contact surfaces such as appliance exteriors, baskets, bassinets, bathroom fixtures, bed rails, cabinets, carts, cell phones, chairs, child-restraint seats, computer keypads, computer screens, counter tops, diaper pails, drawer pulls, door knobs and handles, exercise and weight-lifting equipment, flooring, incubators, lighting fixtures, office equipment, physical therapy equipment, shopping carts, shower stalls, sinks, stools, tables, telephones, toilet exteriors, toilet seats, trash cans, vanities, and walls. The label indicates that the product may be used on hard, non-porous surfaces including: Formica, glass, and stainless steel. Directions on the proposed label provided the following information regarding use of the product as a disinfectant against viruses: Thoroughly wet the surface with the towelette product. Allow to remain wet for 1 minute.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Batch replication for modified tests: Where batch replication has already been performed and accepted for a product registration with unmodified tests by the recommended methods, additional testing at the same use concentration under modified conditions (e.g., different exposure period, presence of organic soil or hard water, porous surface carriers, etc.) may be conducted with reduced batch replications as followed: For basic efficacy claims (e.g., sterilants, disinfectants, sanitizers), two samples, representing two different batches, instead of three; For supplemental efficacy claims (e.g., fungicides, virucides, and tuberculocides, one sample instead of two.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the

specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi): Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test. Performance requirements for this test: the highest dilution that kills all fungal spores is the minimum effective concentration. Alternatively, the AOAC Use Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. The inoculum employed should be modified to provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum. Ten carriers on each of 2 samples representing 2 different batches must be employed in the test. All fungal spores on all carriers/slides should be killed within ten minutes or less.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 475543-01 "Fungicidal Germicidal Spray Method against *Candida albicans* (ATCC 10231)" by Anne Stemper. Study conducted at ATS Labs. Study completion date – June 30, 2008. Project Number A06303.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots (Lot Nos. 07K29E and 08B06B) of the product, DisCide Ultra Disinfecting Spray, were tested according to ATS Lbs protocol number PAL02040308.FGS (copy provided). The product was received ready-to-use, as a trigger spray. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per product lot were inoculated with 0.01 mL of a conidial suspension of the test organism. The carriers were dried for 30 minutes at 25-30°C at 68% humidity. For each lot of product, carriers were sprayed with the product 3 times at a distance of 6-8 inches from the carrier surface. Each carrier remained exposed to the product for 1 minute at 22.0°C at 29% relative humidity. Following exposure, the remaining liquid was drained off. Individual carriers were transferred to 20 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The carriers were transferred to secondary subcultures of 20 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 at least 30 minutes after the first transfer. All subcultures were incubated for 2 days at 25-30°C. The agar plate subcultures were incubated for 44-76 hours at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The average reported initial colony forming units per carrier, for the test microorganism, is: ***Candida albicans*** **6.65×10^5**.

2. MRID 475543-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A (H3N2) virus (Avian Reassortant)," for DisCide Ultra Disinfecting Spray, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – January 3, 2008. Project Number A05570.

This study was conducted against Avian Influenza A (H3N2) virus (Avian Reassortant; ATCC VR-2072; Strain A/ Washington/897/80 X Mallard/New York/6750/78) using cultures of Rhesus monkey kidney (RMK) cells (obtained from Viromed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. One lot, no. 07K29E, of the product, DisCide Ultra Disinfecting Spray, was tested according to ATS Labs Protocol No. PAL02102607.AFLU (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 51% relative humidity. One dried virus film was sprayed (8 sprays) with the product at a distance of 6-8 inches from the carrier surface. One virus film was exposed to the product for 30 seconds at 20.0°C. After exposure, the plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 8 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.5 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥3.0 $\log_{10}$** for the batch.

3. MRID 475543-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus" for DisCide Ultra Disinfecting Spray, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – January 4, 2008. Project Number A05572.

This study was conducted against Influenza A virus (Strain Hong Kong; ATCC VR-544) using cultures of Rhesus monkey kidney (RMK) cells (obtained from Viromed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. One lot, no. 07J12D, of the product, DisCide Ultra Disinfecting Spray, was tested according to ATS Labs Protocol No. PAL02102607.FLUA (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 51% relative humidity. One dried virus film was sprayed (8 sprays) with the product at a distance of 6-8 inches from the carrier surface. The virus film was exposed to the product for 30 seconds at 20.0°C. After exposure, the plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **4.0 $\log_{10}$**. Taking the cytotoxicity and

neutralization control results into consideration, the average reduction in viral titer was **≥3.0 $\log_{10}$** for the batch.

4. MRID 475543-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus" for DisCide Ultra Disinfecting Spray, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 29, 2008. Project Number A05571.

This study was conducted against Human Coronavirus, ATCC VR-740, Strain 229E, using cultures of WI-38 (human lung) cells (ATCC CCL-75) as the host system. One lot, no. 07K29E, of the product, DisCide Ultra Disinfecting Spray, was tested according to ATS Labs Protocol No. PAL02103007.HCV (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 55% relative humidity. One dried virus film was sprayed (6 sprays) with the product at a distance of 6-8 inches from the carrier surface. One virus film was exposed to the product for 30 seconds at 21.0°C. After exposure, the plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. WI-38 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 9 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.5 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥3.0 $\log_{10}$** for the batch.

5. MRID 475543-05 "AOAC Germicidal Spray Method against *Proteus mirabilis* (ATCC 9240)" by Becky Lien. Study conducted at ATS Labs. Study completion date – June 18, 2008. Project Number A06301.

This study was conducted against *Proteus mirabilis* (ATCC 9240). Two lots (Lot Nos. 07K29E and 08B06B) of the product, DisCide Ultra Disinfecting Spray, were tested using ATS Labs Protocol No. PAL02040308.GS (copy provided). The product was received ready-to-use, as a trigger spray. . Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per product lot were inoculated with 0.01 ml of a 48-54 hour broth culture of the test organism. The carriers were dried for 30 minutes at 35-37°C at 28.1% relative humidity. Each carrier was sprayed with the product 3 times from a distance of 6-8 inches. The carriers were allowed to remain wet for 1 minute at 22°C. Excess liquid was allowed to drain and the carriers were transferred to tubes of 20 ml Letheen Broth containing 0.07 % Lecithin and 0.5% Tween 80 to neutralize. The carriers were transferred to secondary subcultures of 20 ml Letheen Broth containing 0.07 % Lecithin and 0.5% Tween 80at least 30 minutes after the first transfer. All subcultures were incubated for 44.5 hours at 35-37°C, and then examined for the presence or absence of visible growth. Controls included viability, dried carrier counts, neutralizer effectiveness, confirmation of challenge organism, and sterility. The reported average colony forming units per carrier, for the test microorganism, is: ***Proteus mirabilis*** **1.8×10^4**

V. RESULTS

MRID #	Organism	No. Exhibiting Growth/ Total No. Tested		Carrier Population Count (CFU/carrier)
		Lot no. 07K29E	Lot no. 08B06B	
475543-01	*Candida albicans*	1°= 0/10 2°= 0/10	1°= 0/10 2°= 0/10	2.5×10^4
475543-05	Proteus mirabilis	1°= 0/10 2°= 0/10	1°= 0/10 2°= 0/10	1.8×10^4

MRID Number	Organism	Results			Dried Virus Count ($TCID_{50}$/0.1 mL)
			Lot No. 07K29E	Lot No. 07J12D	
475543-02	Avian influenza A (H3N2) virus	10^{-1}-10^{-2} dilutions	Cytotoxicity	-	$10^{5.5}$
		10^{-3} to 10^{-7} dilutions	Complete inactivation	--	
		$TCID_{50}$/ 0.1 mL	$\leq 10^{2.5}$	-	
		TCD_{50}/ 0.1 mL	$10^{2.5}$	-	
		Log reduction	$\geq 3.0 \log_{10}$	-	
475543-03	Influenza A virus	10^{-1} to 10^{-7} dilutions	-	Complete inactivation	$10^{5.5}$
		$TCID_{50}$/ 0.1 mL	-	$\leq 10^{0.5}$	
		TCD_{50}/ 0.1 mL	-	$10^{1.5}$	
		Log reduction	-	$\geq 4.0 \log_{10}$	
475543-04	Human Coronavirus	10^{-1}-10^{-2} dilutions	Cytotoxicity	-	$10^{5.5}$
		10^{-3} to 10^{-7} dilutions	Complete inactivation	-	
		$TCID_{50}$/ 0.1 mL	$\leq 10^{2.5}$	-	
		TCD_{50}/ 0.1 mL	$10^{2.5}$	-	
		Log reduction	$\geq 3.0 \log_{10}$	-	

VI. CONCLUSIONS

1. The submitted efficacy data (MRID 475543-01) **support** the use of the product, DisCide ULTRA Disinfecting Spray, as a disinfectant with fungicidal bactericidal activity against *Candida albicans* on hard, non-porous surfaces at room temperature in the presence of a 5% organic soil load for a contact time of 1 minute.

2. The submitted efficacy data **support** the use of the product, DisCide ULTRA Disinfecting Spray, as a disinfectant with virucidal activity against the following microorganisms on hard,

non-porous surfaces at room temperature in the presence of a 5% organic soil load for a contact time of 30 seconds:

Avian Influenza A (H3N2) virus	MRID 475543-02
Influenza A virus	MRID 475543-03
Human Coronavirus	MRID 475543-04

3. The submitted efficacy data (MRID 475543-05) **support** the use of the product, DisCide ULTRA Disinfecting Spray, as a disinfectant with bactericidal activity against *Proteus mirabilis* on hard, non-porous surfaces at room temperature in the presence of a 5% organic soil load for a contact time of 1 minute.

VII. RECOMMENDATIONS

1. The proposed label claims that the product, DisCide ULTRA Disinfecting Spray, is an effective "one-step" disinfectant against the following microorganisms on hard, non-porous surfaces for a contact time of 1 minute:

Candida albicans
Proteus mirabilis

Data provided by the applicant **support** these claims.

2. The proposed label claims that the product, DisCide ULTRA Disinfecting Spray, is an effective "one-step" disinfectant against the following microorganisms on hard, non-porous surfaces for a contact time of 30 seconds minute:

Avian Influenza A (H3N2) virus
Influenza A virus
Human Coronavirus

Data provided by the applicant **support** these claims.